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The Lower Urinary Tract Science Group – ICI-RS. 5th June 2019

Record of Meeting at Bristol University, UK

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High frequency stimulation of the pelvic nerve or S1 sacral nerve root to inhibit urinary voiding - which is best?

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Introduction and Aims

'On-demand' high frequency (1-3kHz) stimulation of the pelvic nerve (PN) in rats inhibits imminent voids[1,2] and could be an attractive novel therapeutic strategy for controlling urinary urge incontinence in humans. Whilst PN stimulation is effective, translation to the clinic is limited by the invasive surgery required. We therefore investigated whether stimulation of the sacral nerve root containing pelvic nerve afferents, might be a preferable target.

Methods

Urethane-anaesthetised female Wistar rats were instrumented for continuous cystometry and stimulation of the pelvic preganglionic nerve bundle (PN) and S1 spinal nerve root; the level at which PN afferents enter the cord in rats. Saline was infused into the bladder (6ml hr^{-1}) to evoke repeated voids.

Results

When initiated at the onset of the steep rise in bladder pressure signalling an imminent void, stimulation of PN or S1 (1-3kHz, 1mA for 60s) suppressed the void. Whilst stimulation at both PN and S1 was effective, PN stimulation was accompanied by transient rises in blood pressure and abdominal pressure and contraction of abdominal muscles, which were not evoked by S1 stimulation.

Conclusions

High frequency stimulation at PN and S1 are both effective in suppressing imminent voids. However, ease of surgical access to S1 and minimal side-effects produced by stimulation compared to PN, combined with extensive clinical experience with sacral neuromodulation techniques is preferable. This indicates that high frequency S1 stimulation may be a better target for developing suppression of unwanted voids 'on-demand' to manage urinary urge incontinence.

Supported by MRC. Grant G1002251 and EPSRC-NIHR HTC Partnership award (IMPRESS) EP/M000109/1

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Neural network analysis to assist differentiation of male outlet obstruction from underactivity

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Introduction and Aims

Detrusor underactivity (DU) and bladder outlet obstruction (BOO) are two prevalent urinary dysfunctions in males. Currently the only standardised methods for diagnosis is pressure-flow studies, but the similarity of low a flow rate in each means that non-invasive methods cannot differentiate between the two conditions. Several studies have indicated that uroflowmetry parameters could serve as additional indicators for differentiation, however poor accuracy in individual assessments hampers their possible clinical use. In this analysis, an artificial neural network is assessed by employing non-invasive parameters to differentiate between DU from BOO.

Methods

The parameters employed are same as in the previous statistical model[1]. The neural network was designed with one hidden layer to avoid overfitting, and the number of hidden neurons was chosen by five cross-fold validations. The data cohort contained free-flow data in 293 males, 135 with DU and 158 with BOO. These were divided randomly into 70% for training, 15% for validation and 15% for testing.

Results

The designed neural network yielded 79.5% overall diagnosing accuracy with 75.6% sensitivity and 82.9% specificity to differentiate between DU with BOO. The diagnosing accuracies in training, validation and testing were 79%, 77.3% and 84.1% respectively.

Conclusions

The results show neural network analysis is more robust compared to other linear models, for instance multivariate analysis. This initial trial employed a feed-forward neural network to assess its ability to assist non-invasive differentiation between DU from BOO by employing free-flow derived parameters. Further analysis will increase the data number, assess convolutional or feed-forward neural networks, and include data from normal individuals for non-invasive diagnosing.

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Urofacial syndrome and innervation of the bladder outflow.

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Introduction and hypothesis

Urofacial syndrome is an autosomal recessive disease characterised by bladder dysfunction with a high-pressure bladder and outflow dyssynergia. If untreated it leads to kidney failure due to urine reflux from the bladder to the kidney. We found that individuals with urofacial syndrome carry homozygous null mutations in either *heparanase 2* (*HPSE2*) or *leucine-rich repeats and immunoglobulin-like domains 2* (*LRIG2*). Until recently, nothing was known about the pathophysiology underlying urofacial syndrome. The aim of this study was to define the bladder defect in mouse models of the disease.

Methods

Analysis of *Hpse2* and *Lrig2* homozygous mutant mice. Voiding stain on filter paper analysis of mouse voiding patterns. Whole tissue bladder immunostaining and confocal imaging; bladder body and outflow physiological analyses using isolated tissue myography with chemical and electrical field stimulation.

Results

The genetic mouse models of urofacial syndrome had urination defects resembling those in human patients. Immunostaining whole bladders revealed a patterning defect of the nerves that control urinary voiding.[1] Physiological analysis of the bladder body revealed a hypercontractile response to the muscarinic receptor agonist carbachol, with no change to relaxation profiles no relaxation of contraction defect was observed in the outflow.

Conclusion

Collectively, these results provide compelling evidence that a peripheral neuropathy underlies bladder dysfunction in urofacial syndrome. Further investigation is required to reveal whether the hypercontractile muscarinic response in the body is a primary or secondary phenomenon, and whether the neuropathy affects the coordination of bladder body contraction and outflow relaxation.

Supported by Kidney Research UK Grant PDF_005_20151126

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A proposal for non-invasive abdominal straining measurement combined with uroflowmetry

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Introduction and Hypothesis

Differentiating bladder outlet obstruction (BOO) from detrusor underactivity (DU) in symptomatic men requires invasive urodynamics. An analysis of a large urodynamic database [1] showed a significant proportion of underactive patients describe straining during voiding, whereas an earlier study [2] showed obstructed men gain little benefit from straining. We therefore aim to examine whether the effect of abdominal straining on urine flow patterns can diagnose each group noninvasively. We hypothesise that an obstructed man will not by straining vary the urine flow greatly, due to the strain increasing the obstructive effect of an enlarged prostate.

Methods

Thirty-two male patients (16 BOO, 16 DU) will be recruited and asked to void twice, once while not straining and once while straining. During each void, abdominal pressure will be measured to identify the straining episodes, using a rectally placed water-filled catheter. In addition, simultaneous measurement with a tocodynamometer and abdominal EMG surface electrodes will be made, to identify whether these methods will reliably and acceptably quantify the strains, so future studies can be done without the rectal catheter. Comparison will be made of the ability of each method to detect straining and the variations in urine flow caused by straining. Ethical approval has been granted for the study.

Conclusions

This study will test the hypothesis that straining during voiding affects urine flow differently, depending upon whether the male patient is obstructed or underactive. The prospect of less invasive, quicker and cheaper diagnosis will benefit both patients and healthcare providers.

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Expression of a transcription factor protein in human prostate cancer.

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Introduction and Aims

Prostate cancer (PCa) is the most commonly diagnosed cancer in UK men and Early detection is key to reducing mortality rates. Therefore, there is a need to develop quantitative and reliable clinical biomarkers for its early detection and prognostication. We have previously shown that the Wnt signalling pathway is dysregulated in PCa.[1] In this study we investigated the expression of the transcription factor, Pygopus-1, chosen for its association with the Wnt pathway. Transcription factor activation is a key event in carcinogenesis. Here we evaluate the performance of Pygopus-1 as a biomarker of PCa.

Methods

Tissue arrays (TA) were constructed from human archival formalin-fixed paraffin embedded samples, containing areas identified as PCa and PCa-adjacent by an expert pathologist. Using automated immunohistochemical (IHC) techniques,[2] 6µm sections were stained for Pygopus-1 by labelling with 3,3'-Diaminobenzidine (DAB). Slides were scanned using a bright field slide scanner (Hammamatsu) and tissue core images were exported from the scanned slide for quantification.

Results

DAB labelling of Pygopus-1 revealed expression of the transcription factor in both PCa and PCa-adjacent tissue cores. In PCa tissue cores, staining appeared to localise to the acinar cells. Quantification of DAB staining revealed an increase in protein expression of Pygopus-1 in prostate cancer tissue cores ($P<0.0001$, Mann Whitney U).

Conclusions

Initial findings indicate that Pygopus-1 could play a role as a putative biomarker of PCa. Furthermore, a biomarker analysis pipeline is being developed based on the principle of measuring protein expression on stained TA.

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Connexin 43 (Cx43) and Wnt signaling

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Introduction and Hypothesis

Wnt signalling plays a key role during development and disease. The transcription factor β-catenin, intracellular Ca^{2+} are major transducers of Wnt signalling. Wnt signalling activation releases β-catenin which translocates to the nucleus. Connexin 43 (Cx43), a member of the gap junction family is a target of Wnt-induced transcription and regulation.[1] However, the inter-relationship between Wnt signaling and Cx43 protein remains unknown. We hypothesised that Wnt signalling activation modulates Cx43 mobiisation in PC3, a prostate cancer cell line.

Methods

Cell fractionation of PC3 cells with or without Wnt5A was performed using a Qproteome cell compartment kit (Qiagen). Cell fractions were loaded onto an SDS PAGE gel and probed with Cx43 antibody using Western blotting;[2] signals were quantified with ImageJ.

Results

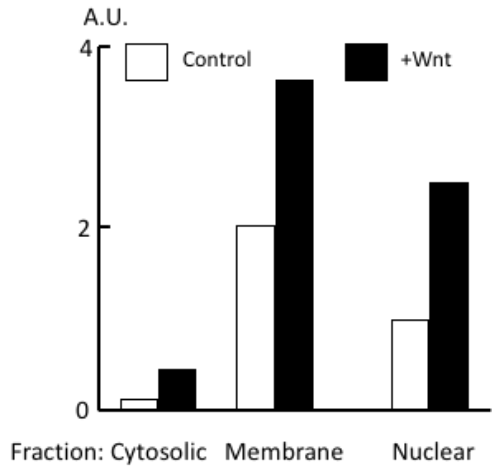

Figure 1 shows an increase of Cx43 signal in the nuclear and membrane fractions after Wnt treatment. Similar results were obtained for another Wnt ligand, Wnt 9B.

Figure 1. Quantification of the intensity of Cx43 expression signal from Western blots in PC3 cells with Wnt5A treatment (black bars) and in control untreated cells (white bars); A.U., arbitrary units.

Conclusion

In response to Wnt signalling activation triggers intracellular mobilisation of Cx43, in a similar manner to translocation of β-catenin and may suggest a cross-talk between Wnt signalling and gap junction proteins.

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Psychological stress and bladder function

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Introduction and Hypothesis: Stress greatly influences the development of bladder symptoms,[1] but the mechanisms involved are little understood. This study investigates the hypothesis that psychological stress in mice induces bladder dysfunction via altered contractile mechanisms.
Methods: Female mice were placed on a pedestal surrounded by water for 1hr/day for 10 days, to induce water avoidance stress (WAS). Controls were not exposed to stress. 24-hours after the final stress exposure, or following 10 days recovery, animals were euthanised, a blood sample taken for a corticosterone assay and whole bladders were isolated, catheterised and intravesical pressure recorded.
Results: Plasma corticosterone levels and voiding frequency were increased in the WAS group compared to controls (Figure 1). Bladders from stressed mice showed greater contractility in response to carbachol ($p<0.05$) and to the purinergic agonist ATP (1mM, $p<0.05$) compared to controls. Voiding frequency was reduced following 10-days stress-free recovery (Figure 1B). This was accompanied by an increase in bladder compliance (Figure 1C), which had been unchanged during the stress protocol.

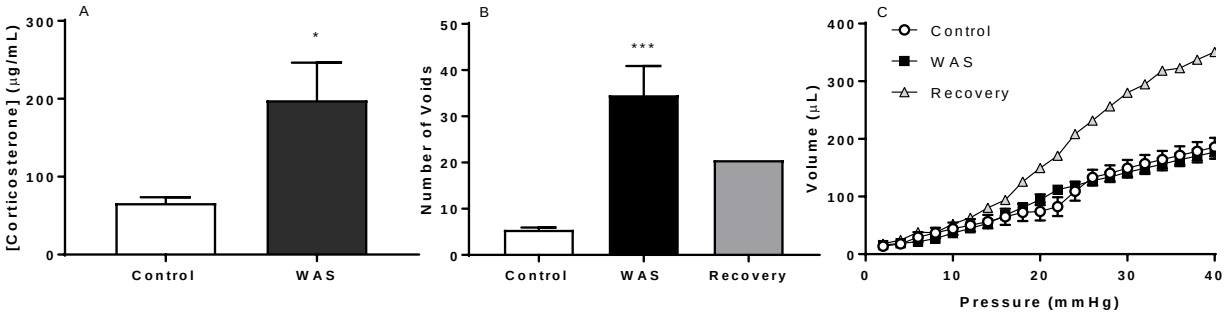

Figure 1: A) Plasma corticosterone levels in control and WAS mice; B) number of voiding events during a 4-hour observation and C) bladder compliance in control, WAS and Recovery mice.

Conclusions: Repeated exposure to environmental stress produces a hormonal stress response and an overactive bladder phenotype. A stress-free recovery period reduced voiding frequency, but this was due to compensation via increased bladder compliance, rather than a reversal of the stress-induced changes.

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Heterogeneity of PDGFRα(+) cells in the urogenital system

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Introduction and AIms

PDGFRα(+) cells in the gastrointestinal tract are considered to suppress muscle excitability by generating hyperpolarising signals[1]. PDGFRα (+) cells are also distributed in the bladder and generate SK3 outward currents in response to P2Y1 stimulation[2]. However, a functional coupling of PDGFRα(+) cells with detrusor smooth muscle (DSM) cells has not been demonstrated. We have examined whether PDGFRα (+) cells in other regions of the urogenital system have similar properties.

Methods

PDGFRα-GFP mice were used and the distribution of PDGFRα (+) cells and their SK3 expression were investigated. Ca^{2+} dynamics in PDGFRα (+) cells were also visualized using Cal-590 fluorescence.

Results

In the bladder, PDGFRα(+) cells in DSM but not mucosal layer expressed SK3 immunoreactivity. PDGFRα (+) cells in the both layers develop P2Y1-mediated Ca^{2+} transients. However, these Ca^{2+} transients were not associated with inhibition of Ca^{2+} transients in DSM cells. In the renal pelvis, atypical smooth muscle cells (ATSMCs) expressed weak PDGFRα-GFP fluorescence compared with two other distinct populations of PDGFRα(+) cells, but appear not to be directly activated upon P2Y1-stimulation. In the seminal vesicle, PDGFRα(+) cells in the musculature but not mucosa expressed SK3 immunoreactivity, while PDGFRα(+) cells in both layers responded to P2Y1-stimulation.

Conclusions

PDGFRα(+) cells in the urogenital system display a heterogeneity in terms of their SK3 expression and P2Y1 response. There was no evidence that PDGFRα(+) cells suppress the excitability of DSM cells, while PDGFRα(+) ATSMCs generate depolarising rather than hyperpolarsing signals.

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The distribution of aquaporins in the pig bladder wall and its relevance to water movements

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Introduction and Aim

Bladder urothelium expresses aquaporins (AQPs) with AQP1-4, AQP7, AQP9 and AQP11 found in various species suggesting they could regulate urothelial cell volume and water transport.[1,2] The aim was to investigate AQP expression and function in porcine bladder, an alternative to human bladder.

Methods

Mucosa (urothelium/suburothelium) was dissected from female pig (*Sus scrofa*) bladders (≈6 months) obtained from the local abattoir. AQP1-11 transcription and protein expression were tested by qPCR and Western blot. Localisation was by immunohistochemistry, using an avidin-biotin-peroxidase system. Water (deuterium oxide, D_2O) apical-to-basolateral flux was measured across a mucosa sheet in an Ussing chamber, ±the non-selective AQP inhibitor, $HgCl_2$ (300 µM), starting apical concentration 40% v/v. Data are mean±SEM, significant ($p<0.05$) differences between sets were determined by Student's *t*-tests.

Results

AQP1,3,9,11 mRNA and protein expression were found in the mucosa, with AQP3,9,11 localised to urothelium and AQP1 to blood vessels. There was a constant apical-basolateral D_2O flux when the apical side was exposed to hypertonic or hypotonic solutions. The average diffusion rate for D_2O in the absence of $HgCl_2$ (0.41±0.06 $D_2O\%.hr^{-1}.cm^{-2}$, $n=9$) was significantly ($p<0.01$) greater than with $HgCl_2$, 0.20±0.02 $D_2O\%.hr^{-1}.cm^{-2}$), with flux rates greater in hypotonic compared to hypertonic media.

Conclusions

The demonstration of AQPs in pig bladder urothelium and a significant $HgCl_2$-sensitive water flux, dependent on the osmotic gradient, shows a role in regulating net water flux from urine to blood. The magnitude of water flux and if this can significantly alter bladder urine osmolality remains to be determined.

Supported by a fellowship from Ferring Pharmaceuticals

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Bladder contractile function in children with congenital bladder anomalies

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Introduction.
Several congenital anomalies are associated with poor bladder function, even after successful surgical correction of the defect, with life-long consequences. The causes of diminished contractile function are unknown and we investigated using bladder tissue biopsies retrieved during corrective surgery.

Methods.
Biopsies were obtained from children with: obstructed bladders from posterior urethral valves (*n*=7); exstrophy (*n*=11) or myelomeningocoele (*n*=12). A control group were children with normal bladder function. All tissue was obtained with informed consent and ethical committee approval. Isolated detrusor strips were used to record isometric tension and for histology to measure smooth muscle and connective tissue (van Gieson stain).[1] Intracellular Ca^{2+} was measured in isolated myocytes with the fluorochrome Fura-2

Results.
Contractile function was reduced to a similar extent in all pathology groups compared to control, whether force was generated by EFS or 1 µM carbachol. This was mirrored by an increase of the connective tissue to smooth muscle ratio. With detrusor myocytes intracellular Ca^{2+} responses to carbachol or high-K solutions were similar in all pathology and control groups. Atropine-resistance of EFS contractions was similar in all groups.

Discussion and Conclusions. Reduced contractile function in all three congenital anomalies was mirrored by a reduced smooth muscle content in detrusor samples. However, similar intracellular Ca^{2+} responses to contractile agonists implies no loss of intrinsic detrusor contractility. Data are consistent with increased passive mechanical stiffness in exstrophy tissue.[1] The ubiquitous presence of atropine resistance suggests it is normal in neonatal/ paediatric bladders, as this declines in normal bladders toward adolescence.[2]

Supported by the Royal College of Surgeons of England and the Children's Research Fund, Liverpool.

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Effects of soluble guanylyl cyclase activators on contractile performance

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Introduction and Aims:
Phosphodiesterase type 5 (PDE5) inhibitors like sildenafil alleviate lower urinary tract symptoms[1]. PDE5 inhibitors block cGMP hydrolysis and recently sildenafil was shown to inhibit the purinergic components of nerve-mediated contractions[2]. Soluble guanylyl cyclase (sGC) catalyses cGMP synthesis. The aim of this study was to assess the effects of an sGC activator BAY 58-2667 on nerve-mediated contractions and ATP release.
Methods: Isolated intact bladder strips from 12-week old C57BL/6 male mice were tied to isometric force transducers. Nerve-mediated contractions were generated by electrical field stimulation (EFS: 0.1 ms pulses, 1-40 Hz, 3-s train every 90-s), and force-frequency relationships were generated. Nerve-mediated ATP release was measured using a luciferin-luciferase assay[2]. Data are means±SD.
Results: The addition of BAY 58-2667 (10µM, n=6) resulted in the reduction of nerve-mediated contractions (two-way ANOVA, $p<0.05$), from 0.64±0.18 $mN.mg^{-1}$ to 0.40±0.14 $mN.mg^{-1}$ at 2Hz, and from 1.57±0.57 $mN.mg^{-1}$ to 1.61±0.63 $mN.mg^{-1}$ at 20Hz, with an increase in $f_{1/2}$ value from 2.97±1.98Hz to 5.16±1.48Hz (Student's paired t-test, $p<0.01$). BAY 58-2667 reduced nerve-mediated ATP release (two-way ANOVA, $p<0.001$), from 102.3±14.5 pmoles to 59.2±12.0 pmoles at 8Hz stimulation.
Conclusions: The data are consistent with the hypothesis that an increase in cGMP levels in detrusor preparations reduces nerve-mediated contractions and ATP release. Nerve-mediated ATP release is a feature of detrusor contractility from overactive human bladder. The attenuation of the purinergic components of nerve-mediated contractions by sGC activators suggests that modulating cGMP in efferent nerve terminals may be a target to reduce overactive bladder contractions.

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Investigation of P2X7R physiology in urothelial cells

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Introduction and Hypothesis

The bladder expresses a number of purinergic receptors including P2X7R (1,2). P2X7R functions as a non-selective ion channel for influx of Na^+, Ca^{2+} and K^+. P2X7R activation is also associated with formation of large pores, enabling transport of larger molecules. Urothelial cells express P2X7R however, little is known of their physiology. Here, we hypothesised that P2X7R were functional ion channels in urothelial cells.

Methods

Immortalised human urothelial cells, SVHUC, were used in physiological experiments including patch-clamp and fluorescence Ca^{2+}-imaging. P2X7R knockdown was conducted via siRNA molecules and confirmed by Western blotting. YO-PRO-1 dye-uptake experiments were performed to determine whether ATP could evoke pore formation.

Results

SVHUC were patch-clamped with Cs^+-filled electrodes, in Hank's physiological solution at room temperature. Application of ATP (1 mM, to activate P2X7R), evoked inward currents in cells held at -60mV. Inward currents were also evoked by a selective P2X7R agonist, BzATP (0.5mM). SVHUC exhibited Ca^{2+}-transients when exposed to ATP (1 mM) that were reduced by the P2X7R antagonist, A740003 (10 µM). siRNA knockdown markedly reduced P2X7R protein expression; these cells had smaller ATP-evoked Ca^{2+}-responses compared to scrambled controls. Dye uptake experiments indicated that ATP did not evoke pore formation in SVHUC in normal Hank's solution.

Conclusions

The findings support the hypothesis that urothelial cells express P2X7R which function as ion channels when activated with ATP or BzATP. There was no evidence indicating that under these conditions, P2X7R formed large pores in SVHUC.

Funding from the Medical Research Council (MR/M012425/1) is acknowledged.

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Spontaneous ATP transients in guinea-pig detrusor muscle

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Introduction and Hypothesis or Aims

Spontaneous contractions are a feature of the urinary bladder and are greater in pathological conditions, but their origins are unknown. We tested the hypothesis that a contributor is spontaneous release of ATP from within the bladder wall

Methods

Isolated guinea-pig detrusor muscle strips, with the mucosa removed, were superfused with Tyrode's solution at 36°C and tied to an isometric force transducer. An amperometric enzyme-based ATP electrode was placed on the surface of the strip, with a null (enzyme-free) electrode as reference (Sarissa, UK). Electrical field-stimulation (EFS) was used to elicit nerve-mediated contractions. Interventions were added to the superfusate.

Results

EFS generated frequency-dependent contractions and ATP transients that were abolished by 1 μM TTX and greatly attenuated by nifedipine (1 μM). Atropine (1 μM) reduced. Force by about 50% but had no effect on ATP transients. With no EFS stimulation spontaneous contractions developed that were mirrored by ATP transients with a close correlation in respective amplitudes. Tension and ATP transients persisted in the presence of TTX, atropine and nifedipine. Immediately after EFS spontaneous contractions and ATP transients were temporally suppressed and recovered after about 40 s.

Conclusions

We propose that spontaneous contractions arise from ATP release from nerve-terminals, due to their temporary suppression by previous stimulation of motor nerves that releases ATP and generates contractions. The pharmacology of the transient events is different from nerve-mediated contractions suggesting ATP release is not due to excitation of motor nerves.

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The action of calcineurin inhibitors on bladder contractile function and ATP release

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Introduction.
Overactive bladder is characterised by spontaneous contractions. Several hypotheses include: increased functionality between mucosa and detrusor; increased mucosal ATP release and augmented detrusor contractions. Increase of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin (Cn) activity is implicated in the aetiology of defective intercellular signalling in myocardium,[1] but its role in signalling in bladder wall tissues is unknown.
Methods.
Detrusor strips with intact mucosa from *ex vivo* guinea-pig bladders euthanised by cervical stimulation and superfused with Tyrode's solution at 37°C. Carbachol (0.1 μM) contractures or spontaneous contractions were recorded in unstimulated preparations. Stretch-activated mucosal ATP release was measured during a stretch of 20% resting length in superfusate samples with a luciferin-luciferase technique. Human detrusor biopsies from normal and OAB human bladders were analysed for calpain (-5,-13) mRNA levels.
Results.
The Cn-inhibitor cyclosporin-A (CsA, 5 and 10 μM) decreased carbachol contractures to 74.7±4.8% and 75.9±4.8% control, respectively; 1 μM CsA had no significant effect (*n*=6). Spontaneous contractions were also reduced by CsA (1,10 μM; 36.2±2.5%, 39.6±7.3% control respectively, *n*=5). CsA (1μM) reduced ATP release from 145±15 to 75±6 fmol/mg wet weight.
mRNA quantification showed gene expression of calpain-5 and calpain-13 increased 1.77±0.32 and 1.84±0.41-fold respectively (*n*=4).
Discussion and Conclusions. CsA diminished agonist-induced and spontaneous contractions, the latter more sensitive to CsA, and stretch-activated mucosal ATP release. mRNA analyses showed that calpain, which renders Cn constitutively active, was significantly increased in OAB human bladder samples. These demonstrate that Cn can upregulate contractile and ATP-signalling functions and may be augmented in OAB.

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Reference
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